Case Report Rapport de cas

High cardiac troponin I plasma concentration in a calf with myocarditis

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Abstract — A 15-day-old Brown Swiss calf, whose dam had suffered from foot-and-mouth disease, was presented with a history of depression and failure to suckle. The calf had an irregular cardiac rhythm and increased plasma cardiac troponin I (cTnI) detected with a commercial human immunoassay. The calf died the following day and myocarditis was detected. The cTnI assay may be useful in diagnosis of myocarditis in cattle.

Résumé — Concentration plasmatique cardiaque élevée de troponine I chez un veau atteint de myocardite. Un veau de race Suisse Brune âgé de 15 jours, dont la génitrice souffrait de la fièvre aphteuse, a été présenté avec une anamnèse de dépression et d'absence de succion. Le veau avait un rythme cardiaque irrégulier et une concentration de troponine I cardiaque plasmatique élevée (cTnI) détectée à l'aide d'un immunoessai humain commercial. Le veau est mort le lendemain et une myocardite a été détectée. L'essai cTnI peut être utile pour le diagnostic d'une myocardite chez le veau.

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A 15-day-old, Brown Swiss calf was presented to the Firat University Veterinary Teaching Hospital with a history of depression and failure to suckle. The mother of the calf had suffered from foot-and-mouth disease (FMD) for 3 d.

Case description

Upon examination, the calf had a rectal temperature of 40.7°C, a pulse rate of 124 beats/min, and a respiration rate of 36 breaths/min. Auscultation revealed an irregular cardiac rhythm. Brachial and femoral arterial pulses were weak. There were no vesicular or erosive lesions of foot-and-mouth disease (FMD). Blood samples from a jugular vein were collected into tubes containing lithium heparin and ethylene tetra-acetic acid (EDTA) for plasma cardiac troponin I (cTnI) determination and virology, respectively. After RNA was extracted from the blood, a reverse transcriptase polymerase chain reaction (RT-PCR) for FMD was performed (1). The universal primers (2BF; 5′-CAG ATG CAG GAG GAC ATG TC-3′ and 2BR; 5′-AGC TTG TAC CAG GGT TTG GC-3′) used in the RT-PCR were designed for diagnosis of all 7 serotypes of FMD virus (FMDV). Samples of RNA extracted from blood were taken from cattle with no FMD history and were used as negative controls. Sensitivity of the RT-PCR was determined with the FMDV vaccine strain (tissue culture infectious dose50; log $10^{5.2}$; Sap Institute, Ankara, TURKEY) diluted 10-fold with sterile dH_2O. The detection limit of the RT-PCR was found to be approximately 20 virions per reaction. The RT-PCR of the clinical sample detected a 131 base pair product, which is specific for all serotypes of FMDV (Figure 1).

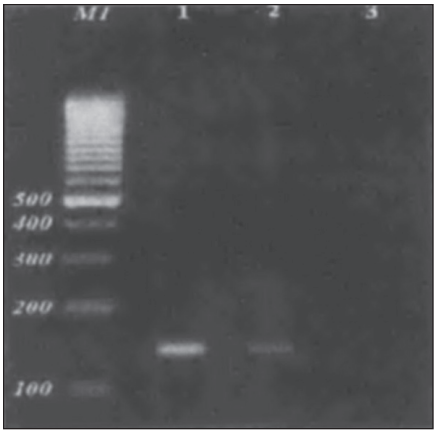

Figure 1. Agarose gel electrophoresis of RT-PCR products. Lane 1 – RT-PCR product amplified from the positive control FMDV vaccine strain. Lane 2 – RT-PCR product amplified from the clinical sample. Lane 3 – RT-PCR product of the negative control RNA from the blood of cattle with no FMD history. M1 – 100 bp DNA Ladder.

Plasma cTnI concentration was determined with a Chemiluminescent Microparticle Immunoassay (CMIA) (Abbot ARCHITECT *i* 2000SR System; Abbott Laboratories, Abbott Park, Illinois, USA) using cTnl-specific purified, monoclonal mouse antibodies that recognize the stable portion of TnI. The detection limit of the assay was 0.01 ng/mL. The plasma cTnI level of the calf was determined to be 37.24 ng/mL. Because no reference ranges for plasma cTnI have been established in cattle, plasma cTnI concentrations from 4 clinically healthy

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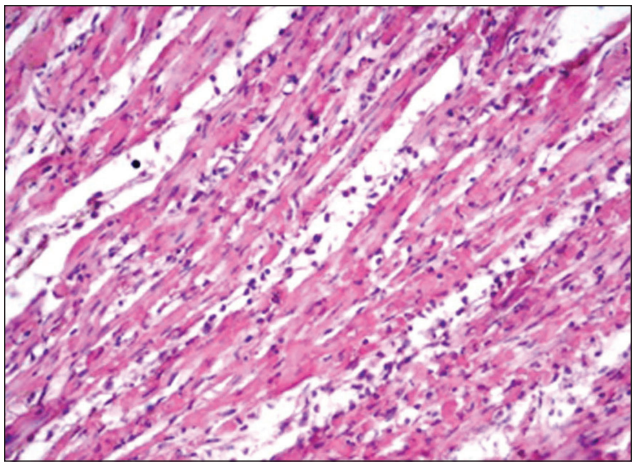

Figure 2. Appearance of inflammatory cell infiltration in the myocardium. Hematoxylin & eosin × 20.

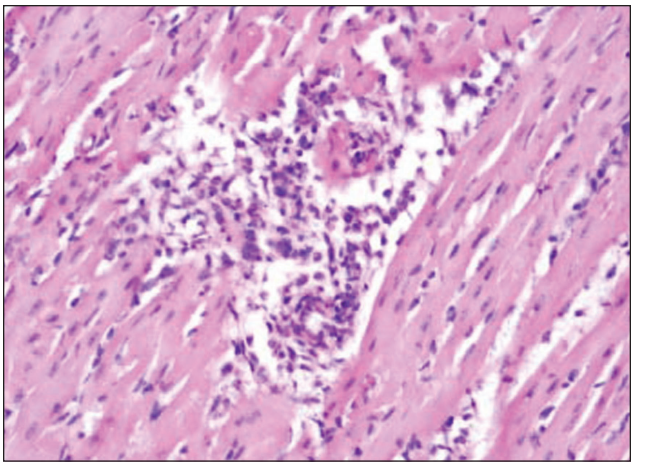

Figure 3. Large numbers of infiltrating inflammatory cells in the perivascular area. Hematoxylin & eosin × 40.

calves were used as control values and were 0.01, 0.01, 0.02, and 0.02 ng/mL. The calf died the following day and a postmortem examination was carried out. Heart tissue from the calf was fixed in 10% formalin and processed by routine techniques. Paraffin sections (5 μm) were stained using hematoxylin & eosin (H&E) and Masson trichrome. Histological sections were assessed and photographed with a photomicroscope (Olympus BX 50). Histologic examination revealed myocarditis in the heart tissue sections. There were multi-focal myocardiocyte swelling, hyaline degeneration, necrosis of myocytes, inflammatory cell infiltration, and inflammatory cell aggregation (Figures 2 to 4).

Discussion

In cattle, myocardial damage may occur due to hereditary factors, vitamin E and selenium deficiency, monensin toxicosis, *Clostridium chauvoei* and *Haemophilus somnus* infection, or FMD (2–5). The dam of the calf had suffered from FMD; consequently, a presumptive diagnosis of myocarditis due to FMD was made although the calf had no vesicular and erosive lesions of FMD. Both myocarditis and FMD were confirmed based on histology and virology, respectively. Histological findings were consistent with the results of other studies reporting myocardial necrosis, hyaline degeneration, and an intense mononuclear cell infiltration (6,7).

There are no specific biomarkers for the diagnosis of myocardial injuries in bovine medicine. Although creatine kinase, lactate dehydrogenase, and their isozymes in humans and animals can be used for detection of myocardial damage, effective use of these enzymes is limited because of lack of tissue specificity and sensitivity (8,9). Myoglobin is increased with both cardiac and skeletal muscle damage; therefore, its use as a biomarker for cardiac disease is limited (8). The best cardiac biomarker for myocardial damage is cardiac troponins (I or T) because they have nearly absolute myocardial tissue specificity and higher sensitivity than creatine kinase, lactate dehydrogenase, and their isozymes and myoglobin (8,10–13). The American College of Cardiology and The European Society of Cardiology have declared cardiac troponin as the preferred biomarker for myocardial infarct (10).

The troponins are proteins integral to the function of striated muscles. They exist as a complex with actin and tropomyosin on the thin filament of the contractile apparatus (12). Following myocardial damage in humans, cardiac troponins leak rapidly from the myocyte and appear in blood after 2 to 4 h and persist up to 10 to 21 d (14). Although the time course of troponin release in response to myocardial damage has not been determined in cattle, a significant increase in plasma cTnI concentration at 1 d after myocardial damage and a gradual reduction to physiological levels over the next 14 d occur in sheep (15). cTnI appears to be more specific than cTnT to detect myocardial diseases because cTnT has also been found to be re-expressed in the skeletal muscles of patients with renal failure and muscular dystrophy (11,12,16–18). No assay has been produced specifically for the detection of cTnI in the serum/plasma of cattle, sheep, horses, or dogs. In this case, plasma cTnI level was measured using the test produced for use in human beings. The antigenic similarity of human and bovine TnI is 96.4% (19). The reactivity of bovine cTnI in human cTnI assays is reportedly higher than the reactivity of equine and sheep cTnI, but lower than the reactivity of dog cTnI (19). Increased serum cTnI levels ranging from 4.3 ng/mL to 5.9 ng/mL have been reported in a horse with multifocal myocardial necrosis (20). In another report, serum cTnI concentration was determined as 404 ng/mL in a horse with severe chronic focal myocardial coagulation necrosis (21). Increased plasma cTnI levels were also determined in canine babesiosis (18). Diniz et al (22) indicated that dogs with ehrlichiosis had higher blood concentration of cTnI than clinically healthy dogs. They determined high cTnI concentration in dogs with ventricular premature complexes. Significantly higher serum cTnI concentrations (24.9 ng/mL) have been reported in dogs that died due to gastric dilatation-volvulus than dogs that survived (2.05 ng/mL), and myocardial damage was confirmed at necropsy (23).

Plasma cTnI levels of sheep were measured at 16.93 ng/mL, 10.12 ng/mL, and 5.46 ng/mL, at 1, 2, and 3 d, respectively after myocardial injury (15). Mellanby et al (24) reported that there is a significant difference between the cTnI concentrations of cattle with pericarditis compared with clinically healthy cattle,

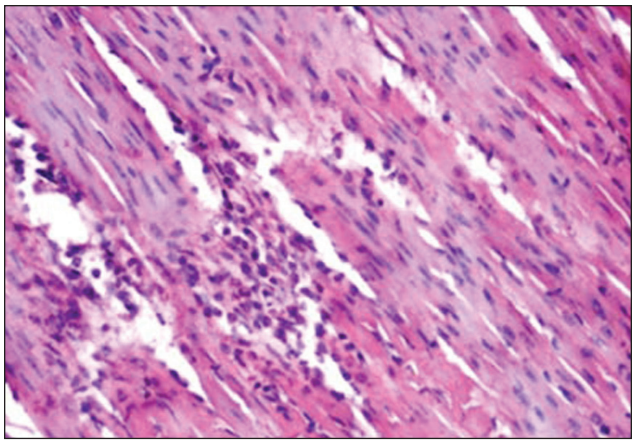

Figure 4. Inflammatory cell aggregation among necrotic cardiac myocytes. Hematoxylin & eosin × 40.

and 4 of the 5 cases of pericarditis had a higher serum cTnI concentration than any of the clinically healthy cattle. Gunes et al (6) determined positive results for cTnI by means of a test kit in a calf with myocarditis, but they did not measure serum or plasma level of cTnI quantitatively. In the present study, plasma cTnI concentrations for the 4 clinically healthy calves were consistent with the values previously established in the literature for healthy large and small animal species (20,25,26). Plasma cTnI concentration of the calf was considerable higher than the values obtained from the clinically healthy calves. The degree of increase in blood cTnI concentration is correlated with myocardial infarct size in humans and dogs because increases in serum troponins following myocardial damage are parallel with decreases in tissue troponin concentrations (11,12,27,28). Highly elevated plasma cTnI concentration in this case may be an indicator of severe myocardial damage and poor prognosis. Results of this case report offer preliminary indications that bovine cTnI may be used as a blood-based biomarker of cardiac disease in cattle, and that the commercial human cTnI assay may be used to diagnose myocarditis in cattle in the same way it is used to diagnose myocarditis in dogs, horses, and sheep. Further controlled studies are necessary to determine the relationship between myocardial damage and cTnI level in cattle. CVJ

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